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Cercarial Parasitism of Freshwater Snails in Sandhills Ponds versus Streams

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TREMATODE CERCARIAL PARASITISM OF FRESHWATER SNAILS IN SANDHILLS PONDS VERSUS STREAMS

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by

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Abstract

Parasite prevalence was calculated for all aquatic snails collected from four freshwater localities in Western Nebraska from Summer 2016 to 2019: Dunwoody Pond, Haythorn Ranch, Neven's Restoration, and Breen's Flyaway. With data of increasing trematode cercarial infection specifically at Dunwoody Pond, this community was further studied. In Summer 2019, a total of 217 freshwater snails representing species of *Physa* sp., *Stagnicola elodes* (Say,1821), and *Helisoma anceps* (Menke,1830) were collected from Dunwoody Pond and Dunwoody stream, near Ogallala, Nebraska. Eighty-one snails were collected from Dunwoody Pond, 76 snails were collected 31 meters upstream, and 60 snails were collected 61 meters upstream the pond. All 217 snails were examined, and three different genera of trematode cercariae, representing several species, were identified including: *Echinstoma* spp. *Strigea* sp., and *Halipegus* sp. Due to lack of statistical difference at locations sampled 31 meters and 61 meters upstream from Dunwoody Pond, sample data collected from the stream were grouped as one category "stream" to facilitate comparison of data of stream versus pond. We found there is a significant difference in parasite prevalence in *Physa* sp. snails collected from the pond versus the stream ($p < 0.00001$).

Keywords: trematode, cercariae, parasitology, prevalence, intermediate host

Appreciation

Thank you to Mr. Duane Dunwoody, his family, and farming staff for allowing students to conduct research on their land for several summers. I feel extremely grateful for my Thesis advisor Dr. Scott Gardner; not only has he instilled in me the deserving respect and importance of biodiversity conservation, but he has challenged me to be knowledgeable of all life as a future physician. To Sebastian Botero-Cañola, I thank him for his positive attitude and guidance in classifying each observed parasite species. I appreciate Dayna McCormick, my partner for the Dunwoody Pond project; I wish her luck as she continues her work in the field of parasitology. My colleague Jenny Adams and Dayna's father, Aaron McCormick, helped collect snails. Thank you to the staff and generous donors at Cedar Point Biological Station for availability of the laboratory, library, and equipment. Without my scholarship award, I would not have had this amazing opportunity.

Introduction

Undergraduate students from around the world have ventured to Ogallala, Nebraska, about five hours directly west of the capitol city, to explore and experience biology outside a typical lecture hall. Students of Dr. Scott Gardner's Field Parasitology course design their own research projects in hopes of answering questions about parasites in the area. With data from students since 2016, potential trends in parasite prevalence could be analyzed. Because data has been collected most consistently from Dunwoody Pond and shows increasing trematode parasite prevalence since 2016, this locality was studied further and in greater detail.

Years ago, Nebraska farmer Duane Dunwoody dug a large hole on his property with intentions of raising catfish (Janovy, 1994). Even though he failed as a catfish farmer, Mr. Dunwoody inadvertently invited nature to fill up the hole with water and "whatever life (that) could ride in on the prairie wind" (Janovy, 1994). Life now found here includes aquatic snails, which are often intermediate hosts of trematodes in freshwater ecosystems (Schell,1985). At Dunwoody Pond, located in Keith County of western Nebraska, snails are found both in the pond and in the stream leading into the pond. Because of its proximity to the Cedar Point Biological Station, the unique stream originating from the Nebraska Sandhills flowing in, and a positive trend of trematode cercarial parasitism in the community, Dunwoody Pond was selected as the locality for this research project.

The word trematodes stem from the Greek root "trematos," meaning "pierced with holes." They are multicellular, eukaryotic helminths, which include parasitic worms, flukes, tapeworms, or nematodes. Typically no more than a few centimeters in length, they often have an oral and ventral sucker. Trematodes are classified as acoelomates and triploblasts, meaning

they have no body cavity but have three primary germ layers. Most trematodes expel undigested material through their mouth because they do not have an anus. They respire by simple diffusion of oxygen via its tegument.

According to the Center for Disease Control and Prevention, the basic life cycle of a trematode usually begins as unembryonated eggs are passed from feces of infected definitive hosts to water. There, depending on the species, they mature for three weeks and develop into miracidia. The miracidia then hatch and swim freely, looking to penetrate the first intermediate host - in this case, an aquatic snail. Snails are omnivores and scavengers; eating decaying plants, small dead animals, and algae. Within the snail, sporocysts, and depending on the species, one or two generations of redia give birth to cercariae. Depending on the species of trematode, cercariae may encyst as metacercariae within the first intermediate host, effectively overwhelming the snail's digestive gland, leave the host to penetrate another intermediate host, or penetrate and develop in its definitive host. Finally, the definitive host is infected after consuming metacercariae found within an infected intermediate host. The metacercariae excyst in the duodenum while adult trematodes typically reside and reproduce in the small intestine. There are a huge number of exceptions to this scenario, and again, depends on the trematode species of study.

Parasite prevalence can be affected by host size, host susceptibility, and host age (Pacala and Dobson 1988, Arneberg 2002, Calhoun et al. 2018). "The presence of a parasite is proof the chemical and physical environment provided by the host is adequate to support the parasite, at least temporarily" (Janovy et al., 1992). However, environmental elements also factor into parasite prevalence. Developmental stages of trematodes rely heavily on a stable

water environment to properly mature. Because the physical environment impacts parasite prevalence, the following question can be raised: How would distance along the stream from the pond correlate with parasite populations within freshwater snails?

The primary investigators of this study hypothesize parasite populations experience different limiting factors both upstream and within the pond itself. It was predicted that as the distance of the collection site of snails increases from Dunwoody Pond (upstream), then the average number of parasites observed per snail will decrease. A similar relationship was found by Knight et al. (1980); prevalence in protozoa of Plains killifish was much greater upstream of a diversion dam compared to downstream. The faster-moving water was considered a less suitable habitat for growth of microorganisms. Hence, less parasites would be found within the snails living downstream.

The purpose of this comparative study was to determine if there is an association between levels of parasite prevalence, intensity, and biodiversity in snails collected upstream and from Nebraska's Dunwoody Pond. These trematode species are categorized as obligate endoparasites and exhibit low host specificity. Prevalence of each trematode cercarial species was calculated for each snail species collected. Intensity of infection was ranked qualitatively as High, Medium, Light, or None.

Materials and Methods

Data were collected from freshwater snails from Summer 2016 to 2019, and organized into a repository. Motives for each student's project was ignored so only the objective meaning of data could be utilized. The level of infection of each snail recorded by former students was graded qualitatively according to the scale explained below. Because all students recorded sample data differently, only parasite prevalence could be determined.

Specifically from the personal project of the Summer 2019, freshwater snails of the genus *Physa* and the species *Stagnicola elodes* (Say,1821) and *Helisoma anceps* (Menke,1830) were collected directly from Dunwoody Pond, 31 meters upstream, and 61 meters upstream from the pond. Using a walking measure, each increment of distance was marked with orange construction flags along the stream. Snails were collected from approximately a three-meter radius of the flags. Snails were collected by sweeping an aquatic net through the banks, sifting sand and sediment in a tray, and by fingering through mud on the banks of Dunwoody Pond and the stream. Snails were collected over a two-week period and dissections were completed the day of collection. Snails were stored in small jars, transported to the lab, and carefully crushed using forceps.

For proper identification, snails were compared to (Figure 1 *Physa* sp.*),* (Figure 2 *Stagnicola elodes* (Say,1821)), and (Figure 3 *Helisoma anceps* (Menke,1830)). Additionally, *Physa* sp. are sinistral, meaning they have left-handed apertures (Paraense et al., 2003) while *Stagnicola elodes* (Say,1821) snails are dexteral with right-handed apertures (Burch, 1982).

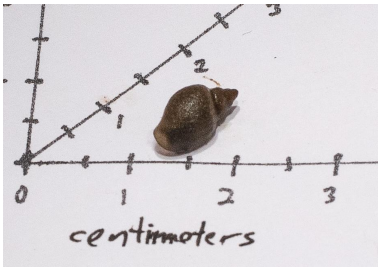

Figure 1. *Physella* sp. snails with left-handed opening.

Figure 2. *Helisoma anceps (Menke,1830)* snails with distinct, circular shell.

Figure 3. *Stagnicola elodes* (Say,1821) snails with right-handed opening.

The contents of the snail's mantle, digestive gland, and digestive tract were separated and examined with a dissecting microscope. Organisms within the snails that proved challenging to identify were placed on a wet mount and photographed with a compound microscope. Parasite specimens were classified according to the morphological guide, "How to Know the Trematodes," by Stewart C. Schell. Characteristics such as tail morphology, location of suckers, and presence or absence of eyespots were used to distinguish between genera of trematode cercariae.

Parasite prevalence was calculated as the number of infected aquatic snails divided by the total number of aquatic snails collected from that area of study or in that sample. Parasite intensity was graded qualitatively, as some snails hosted more than 500 swimming cercariae. Snails categorized as "Light" had less than 50 cercariae or zero to ten sporocysts. A "Medium" level of infection meant the snail had more than 50 but less than 100 cercariae, but ten to 20 sporocysts. Lastly, an aquatic snail had "Heavy" parasite infection if it was host to more than 100 cercariae or more than 20 sporocysts. The number of species observed was recorded as the project progressed.

Results

In analysis of parasite data in aquatic snails since Summer 2016, four localities were found to have large amounts of data: Dunwoody Pond, Haythorn Ranch, Neven's Restoration, and Breen's Flyaway. The map is displayed in Figure 4. In 2018, Haythorn Ranch is reported to have a parasite prevalence of 31.59%, or about 115 of 364 snails. At Neven's Restoration, Summer 2018 had only 1 of 32 snails infected (3.125%) while Summer 2019 presented 25 of 100 snails were infection (25%). At Breen's Flyaway, Summer 2017 had 5 of 9 snails infected (55.55% prevalence), while Summer 2018 had 15 of 110 snails infected (13.63%). From Summers 2016, 2017, 2018, and 2019, Dunwoody Pond had 14 of 214 snails (6.5%), 6 of 9 snails (66.67%), 74 of 206 snails (35.92%), and 124 of 172 snails (72.09%) infected with trematode parasites, respectively. Freshwater snail data were not considered significant from Dunwoody Pond in 2017 and Breen's Flyaway in 2017 for too small of sample size.

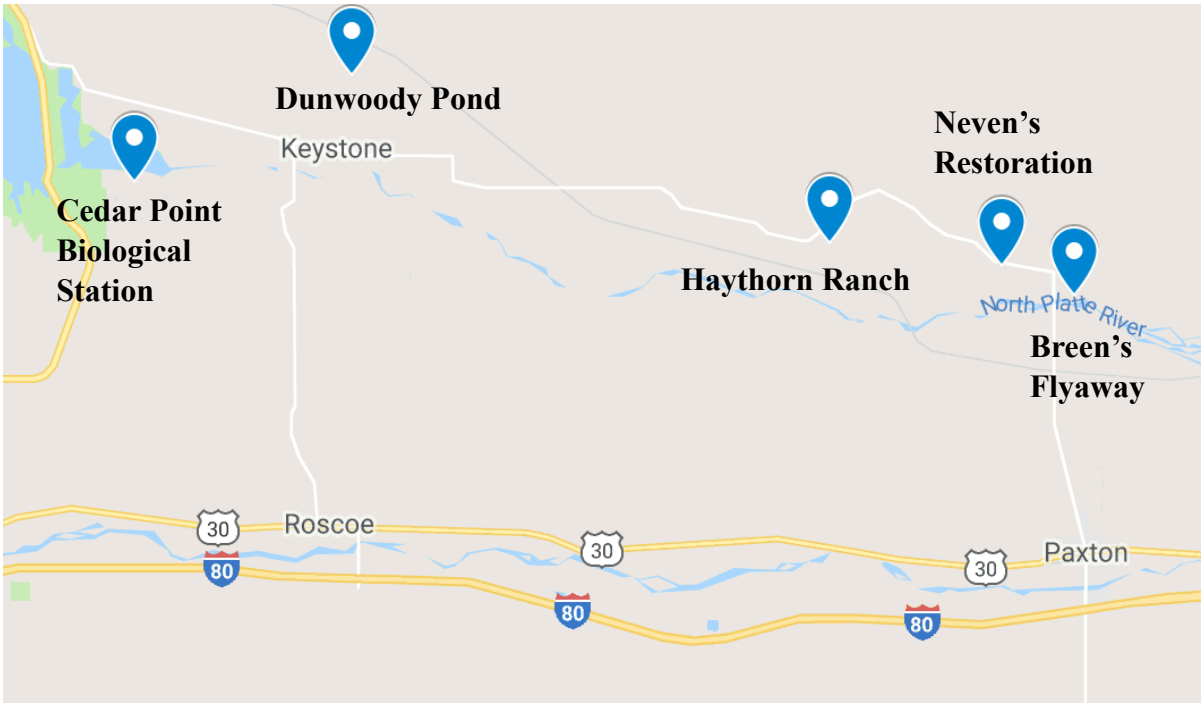

Figure 4. Map of collection sites near Cedar Point Biological Station in Western Nebraska.

In the Summer 2019 Dunwoody Pond project, snails were most abundant at the Pond's edge, closest to the stream's entry, while snails were more difficult to find in the stream itself. Eighty-one snails were collected from Dunwoody Pond, 76 snails were found 31 meters upstream, and 60 snails were found 61 meters upstream from the pond - yielding a total of 217 snails.

Three different genera of trematode cercariae were identified, including *Strigea, Halipegus,* and *Echinstoma*. *Echinostoma* were most prevalent among all three species of snails. All developing stages of trematodes were present in the snails collected from Dunwoody Pond, including: cercariae, metacercariae, redia, and sporocysts. Furthermore, nonparasitic, ectocommensals such as *Chaetogaster* worms and beetle larvae were observed.

Figure 5. *Strigea* sp.

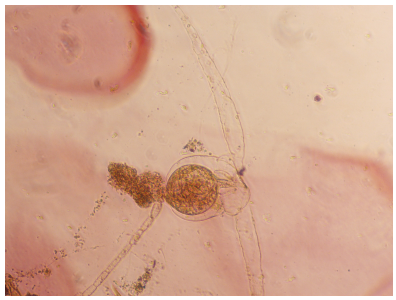

Figure 6(a). *Halipegus* sp.

Figure 6(b). *Halipegus* sp. sporocyst.

Figure 7(a). *Echinostoma* sp. cercariae.

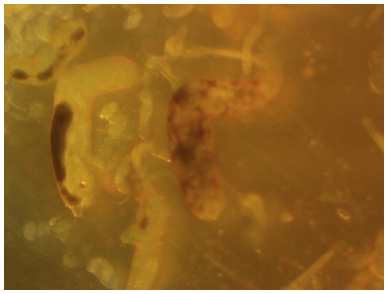

Figure 7(b). *Echinostoma* sp. sporocysts.

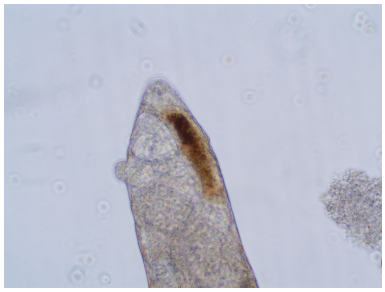

Figure 7(c). *Echinostoma* sp. sporocysts.

By means of a Fischer's Exact Test, it was determined there is no statistically significant difference between parasite prevalence of snails at 31 meters and 61 meters upstream of Dunwoody Pond. Therefore, the researchers decided to group the data observed from the stream as one category.

On the other hand, there is a significant difference in parasite prevalence of *Physa* sp. snails collected from the pond versus the stream ($p < 0.00001$). Additionally, there are signficant differences in parasite prevalence of *Helisoma anceps* (Menke,1830) and *Stagnicola elodes* (Say,1821) snails collected from the pond versus stream with $p < 0.0002$ and $p < 0.0058$, respectively. These values are presented graphically in Figures 8 and 9.

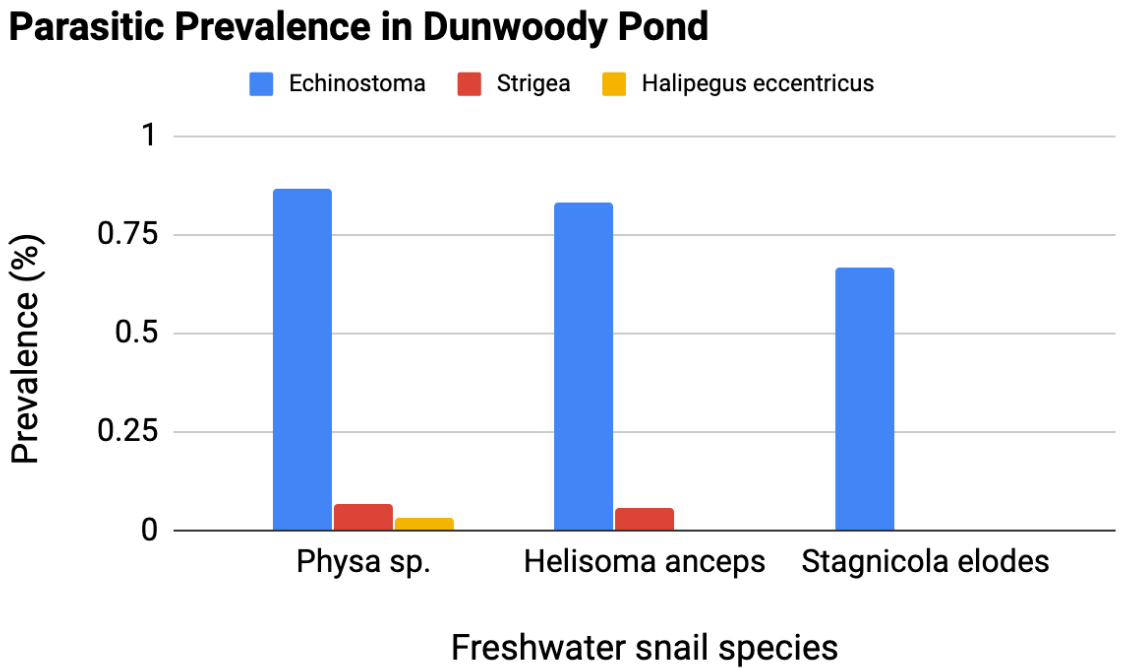

Figure 8: Prevalence of parasites found in snails collected from Dunwoody Pond. *Echinostoma* cercariae were most common among all three snail species.

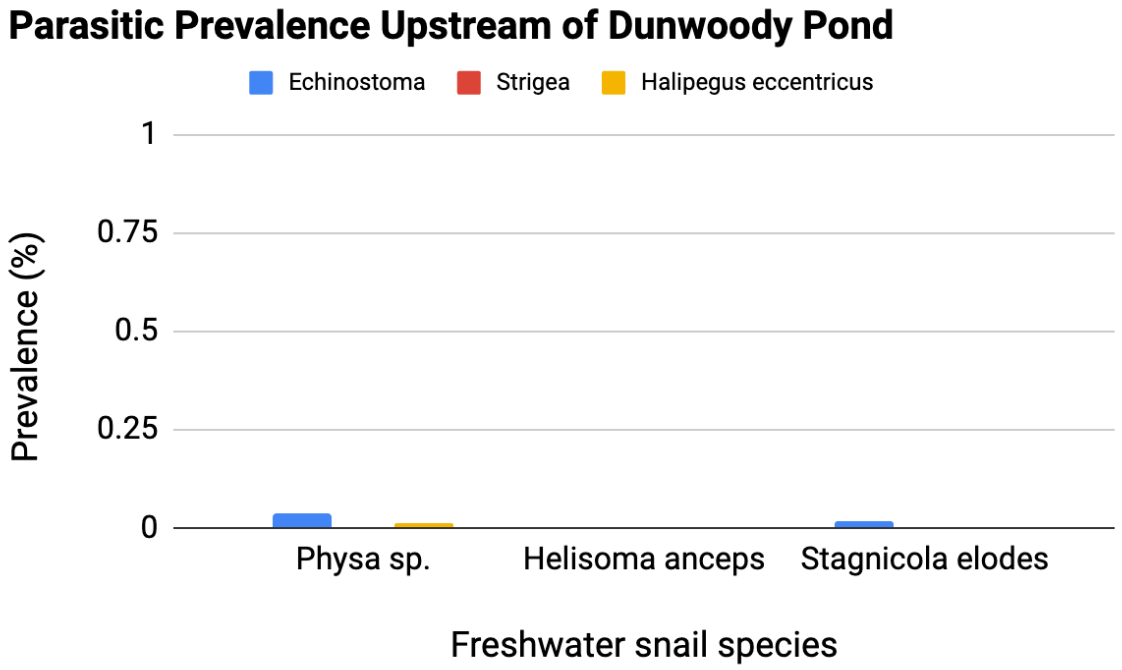

Figure 9: Prevalence of parasites found in snails collected upstream from Dunwoody Pond. Few parasites were observed in stream snails.

An unpaired t-test for comparison of parasite intensity in *Physa* sp. versus *Helisoma anceps* (Menke,1830) snails resulted in a two-tailed p-value equal to 0.0215 and exhibited in Figure 10.

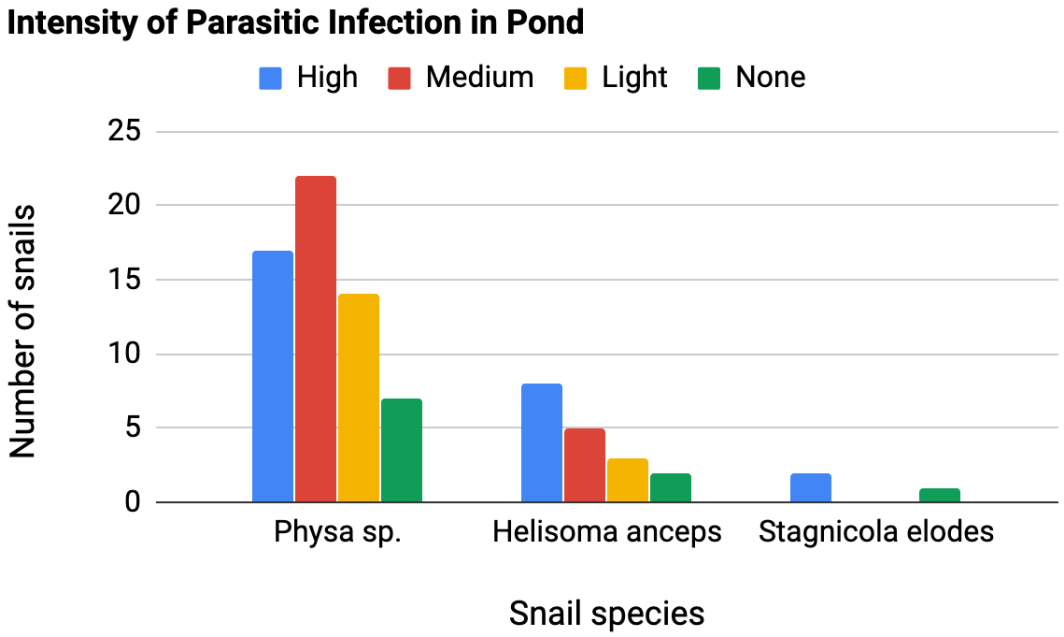

Figure 10: Intensity of parasite infection in snails from Dunwoody Pond. Intensity of infection was graded qualitatively using a scale ranging from High, Medium, Light, and None.

Three different cercariae species were observed in snails collected from the Pond, whereas stream snails only exhibited two different parasite species. This is modeled in the Species Accumulation Curve in Figure 11.

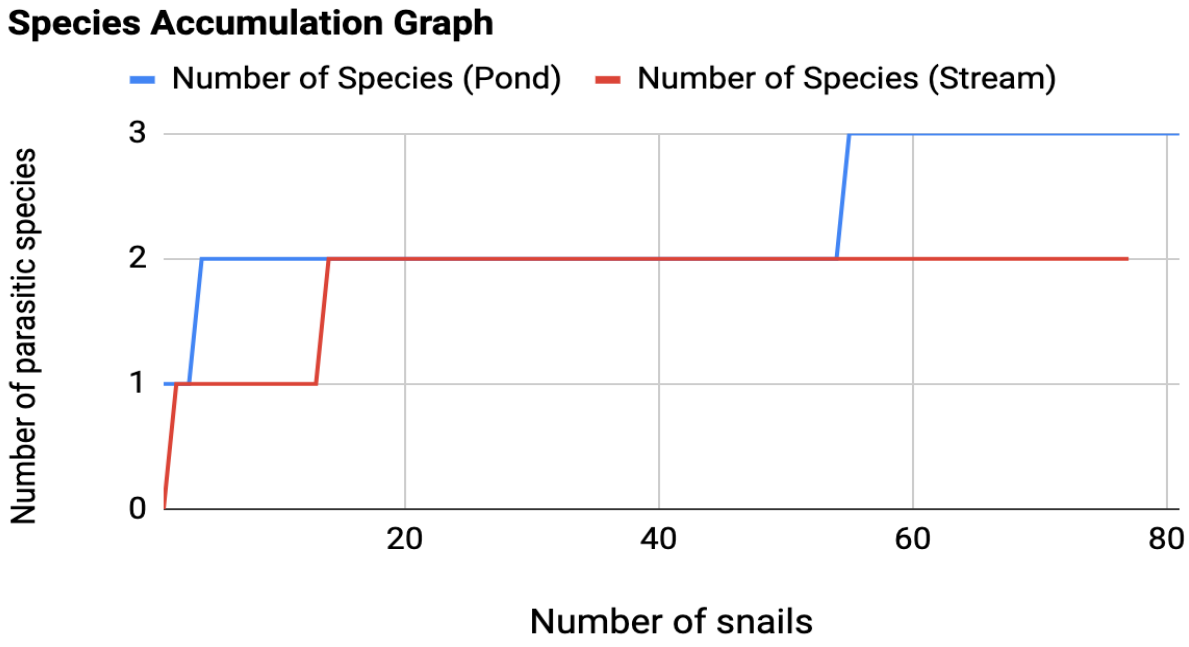

Figure 11: Number of parasite species encountered as snails were analyzed. Three trematode cercariae species were observed in Pond snails, while only two species were found in stream snails.

Interestingly, metacercariae were found congregated in bundles just under the shell, on the mantle of snails only collected from Dunwoody Pond. Observations were recorded and are presented below in Figures 12 and 13. For each snail species collected from the pond, 67% of the *Physa sp*., 61% *Helisoma anceps* (Menke,1830), and 67% of *Stagnicola elodes* (Say,1821) snails contained "clumped" metacercariae.

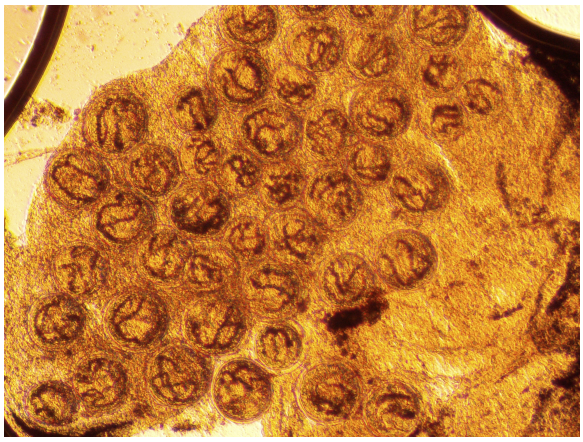

Figure 12. *Echinostoma* sp. metacercariae

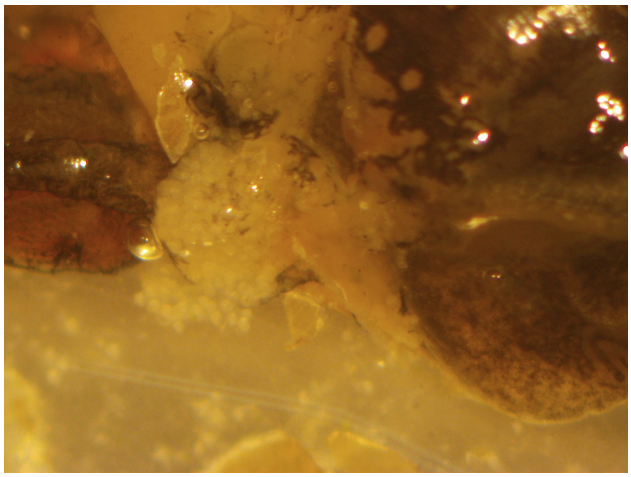

Figure 13. *Echinostoma* sp. metacercariae on mantle of snail.

Discussion

The results of this study demonstrate the importance of change in parasite prevalence between moving and still bodies of water, as parasites were significantly more prevalent in snails for Dunwoody Pond compared to snails from the stream. This serves as justified reasoning for the actions needed to protect our planet's natural streams - to preserve the quality of life reliant upon that water source. This also relates to questions of "healthy" food sources: Is the pond-raised fish I ate last night for dinner more likely to contain parasites compared to the "wild", stream-caught fish?

A limitation to this research project was allotted time. Collection, dissection, literature review, and statistical analysis were all completed in a two-week period. Additional time would have been utilized by the team to explore other pond-stream areas and collect more data. A potentially confounding variable was the presence of a pond several hundred meters upstream from Dunwoody Pond that could affect the parasite prevalence of the Pond and its stream. The researchers advise further investigation of this secondary pond. Also, if the study is to be replicated, one should collect snails on a smaller scale - perhaps 5, 10, 15, and 20 meters away from Dunwoody Pond. This test would potentially better exhibit a gradient of parasite prevalence along the stream.

Regarding long-term data collection at the Cedar Point Biological Station and surrounding areas, the future of student-lead research is bright. There is much more to discover in the plains, ponds, and Sandhills of Nebraska. This repository has been compiled with hope of inspiring other students to build upon past findings. With a better understanding of parasite prevalence in the past few years, questions about phylogeny and behavior may be answered or

even unveil connections between climate change and biodiversity loss. A snail is small, but its sole purpose is not as an intermediate host to trematode cercariae - it has a larger impact on Nebraska ecosystems than most would think.

Several components of this study also open new questions to be investigated. Is there reoccurrence of metacercariae "clumping" in Summer 2020 in response to warmer temperatures? Are the cercariae encysting immediately? Does the width of the body of water affect the probability of where ducks (definitive hosts) land on water? It is also noted Mr. Dunwoody killed all muskrats near his Pond last summer; does this explain the continued increase in trematode parasite prevalence? Even more so, with increasing parasite prevalence at Dunwoody Pond, could trematode redia be genetically modified to "eat" problematic cercariae? Hopefully future students at Cedar Point Biological Station will be inspired to design ways to respond to these unknowns.

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